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Previous in vivo studies left the scientific community with the assumption that rabbits were resistant to prion diseases. However, our recent findings proved they are susceptible. The in vitro results were essential to demonstrate that prion protein (PrP) from every species has the potential to become not only misfolded to a disease associated form, but also capable of being virulent and causing clinical disease. Even though transmissible spongiform encephalopathies have only been described in mammals to date, it would not be too surprising if prion diseases could eventually be found in any class of animal that has PrP such as birds, reptiles or fish.

The first reported observations of a transmissible spongiform encephalopathy (TSE) in Europe were in the first half of the 18th century when Thomas Comber described a disease of sheep, originally called rickets, which we know today as scrapie. However, the ability of TSEs to transmit to other species was unknown until 1960–1970 when the first experimental infections were performed in mice and other laboratory animals.

The differences between TSEs and other contagious diseases were evident early on, starting with the unusual characteristics of their pathogenesis, their unknown origin and especially their ability to be transmitted experimentally to a large number of species, even though different species are not equally susceptible (Barlow et al., Res Vet Sci 1976).

In the 1990s and early 21st century the main aim of TSE research was to establish the etiological agent, and although accomplishing this objective was of fundamental importance, it required most of the available funding resources and thereby prevented investigations of other aspects of these diseases.

Whereas confirmation of the "protein only hypothesis" represented a significant step forward for TSE science, it made the strain phenomenon and transmissibility between species more difficult to explain. Undoubtedly, it would have been easier to explain TSEs if the etiological agent was a virus or a bacterium, instead of one whose principal or only component is a protein.

In the past, many experimental infections were performed using different sources of TSEs, both within and between species, in an attempt to understand their pathogeneses and transmissibilities. However, in many occasions the existence of different strains was ignored.

The appearance of bovine spongiform encephalopathy (BSE) advanced knowledge in this area as a large number of animals were accidentally exposed to a novel TSE agent (Bons et al., Proc Natl Acad Sci U S A 1999). This "unplanned experiment" also showed that not every species was equally susceptible. For example, BSE was found in the goat population in the UK and France, but no cases were reported in pigs, despite proven experimental susceptibility, and having been naturally exposed to the agent during the BSE outbreak. However, we should not generalize with respect to susceptibility to prion diseases as their behaviors, and possibly even their mechanisms, can be as numerous as the number of identified strains. Without knowledge of the intrinsic characteristic of strains, the observation of natural and experimental infections may lead us to think that every strain is a unique and independent agent. This is because, despite having several similar characteristics, sometimes the different TSE strains behave as differently as the influenza virus does from the hepatitis C viruses. For example, different TSE strains target different species and tissues, with different incubation times and result in different clinical manifestations. Due to these innate differences, predicting if a strain will transmit to another species is very difficult and suggesting that a disease associated prion protein generated in cows either can or cannot transmit to humans is also dangerous. At best, we can try estimating the zoonotic potential of animal prions with ad hoc models such as primates or human PrP transgenic mice. Furthermore, it is currently impossible to establish if and how these unconventional agents will adapt and mutate when they infect new species. For all these reasons it is ill-advised to define a species as resistant to prion diseases on the basis of absence of natural cases or experiments where one can only use a limited number of strains.

The degree of pathogenicity of different disease associated prions, or virulence, is determined by the incidence of infection and the length of time between exposure and development of clinical signs. These data allow the classification of prion diseases from low to high virulence, but only if related to a specific species. This is because a TSE which is highly virulent in one species can be of low virulence or even avirulent in another. This paradigm of transmission is influenced by both the TSE strain and the species it is infecting, therefore, it is possible that every species has a specific strain that, once adapted, would represent the most virulent disease associated prion in that species.

The route of infection in TSEs plays a critical role in transmissibility and also the capacity of prions to replicate extraneurally is strain-specific (Beringue et al., Science 2012). This is often also responsible for the virulence of a strain in the same species. An exceptional example of transmissibility is scrapie, which, despite having been recognized for centuries as being highly virulent in sheep and goats, has never been reported as a natural infection in any other species and is therefore considered avirulent in humans. However, no one can be totally sure about its ability to adapt to other species. With respect to this it is important to mention that when BSE has been transmitted to sheep it becomes more virulent on

re-passage as denoted by shorter incubation times in cattle and by an increase in the number of species it is capable of infecting (Padilla et al., PLoS Pathog 2011). These changes could happen with other TSE strains.

It is interesting to consider the potential virulence in common domestic species in which spontaneous prion diseases have never been reported. We should be very cautious in predicting the behavior of TSEs in these animals as transmissibility will depend on the combination of strain and challenged species. Nevertheless one should not consider this area of research an unanswerable enigma unless all strains are tested in every species, as this is unrealistic. To resolve this situation and start addressing some of these questions we have used our expertise in the in vitro replication of prions (Castilla et al., Cell 2005). We have examined a large number of TSE strains/challenge species transmission combinations and performed a two passage study on the susceptibility of rabbits to in vitro generated homologous species disease associated prion infection (Chianini et al., Proc Natl Acad Sci U S A 2012). Prior to this study there were many uncertainties with respect to the susceptibility of rabbits to TSEs; previous in vivo studies had failed to transmit the disease yet the success of our in vitro studies proved that rabbit PrP could be efficiently misfolded after being seeded with different prion strains from different species and even formed an infectious de novo strain from unseeded brain.

The results of the in vivo studies left the scientific community with the assumption that rabbits were resistant to prion diseases. However, our recent findings proved they are susceptible. The in vitro results were essential to demonstrate that PrP from every species has the potential to become not only misfolded to a disease associated form, but also capable of being virulent and causing clinical disease. In our case rabbit PrP misfolded in vitro and produced a de novo protease-resistant PrP from a healthy rabbit brain. This de novo PrP was capable of infecting a small percentage of rabbits on primary passage but a very high percentage succumbed to clinical disease upon second passage. Although with respect to our definition of virulence we could not consider our strain to be highly virulent; the mean for the incubation time was around 550 d post infection, we should not forget that several factors can influence the incubation time without altering the virulence. A clear example of this would be the long incubation times associated with human TSE strains in human infections, which are highly virulent.

From the production of the de novo TSE strain derived from the brain of a healthy rabbit it is tempting to speculate that its formation may be comparable to the spontaneous forms of prion disease, called atypical, and reported in humans and ruminants. These forms of prion diseases have always proven to be efficiently transmitted to the homologous species.

Even though we demonstrated that rabbits are not resistant to prion diseases, the studies performed in vivo previously and then confirmed with our study, showed that this species is not susceptible to TSE strains commonly virulent in other species such as ME7 in mice. These findings highlight the importance of the compatibility between the infectious PrP and the native PrP of the challenged species. This compatibility is dependent upon the amino acid sequence of the PrP and differences between the two proteins can determine the success or not of replication of the disease associated form.

Unfortunately, a simple comparison of PrP amino acid sequences between the species where strains have originated and the ones which are to be investigated cannot determine which PrP amino acids are responsible for successful disease transmission. This is not surprising since different strains with a different clinical course can be raised from the same species which has the same PrP amino acid sequence. To make things more complex, intermediate hosts can change the ability of certain prion diseases to become infectious in a species that otherwise appears not to be susceptible. The mechanism of how this happens is unclear, but the intermediate host may induce a conformational change or a mutation in the disease associated PrP strain or just aid the infectious ability of this strain in a different PrP environment. Therefore, thanks to intermediate hosts, certain TSE strains can increase their virulence and spread to other species as happened for BSE transmitted to sheep as previously explained.

In conclusion, even though TSEs have only been described in mammals to date, it would not be too surprising if, given the chance to evolve through intermediate hosts, prion diseases could eventually be found in any class of animal that has PrP such as birds, reptiles or fish.